PLAN OF THE STUDY : **IMMUNIZATION OF HLA-A1 PATIENTS CARRYING A BREAST CANCER WHOSE TUMOUR EXPRESSES MAGE-1**

1. INTRODUCTION

2. OBJECTIVES OF THE STUDY

3. SELECTION OF PATIENTS

   3.1. Stage of the Disease

   3.2. Inclusion criteria

   3.3. Exclusion criteria

4. THERAPEUTIC SEQUENCE

5. IMMUNIZATION REGIMEN

6. STUDY PARAMETERS

7. IMMUNOLOGICAL STUDIES

8. MECHANICS OF THE STUDY

9. VACCINE SUPPLY

10. ETHICAL CONSIDERATION

11. REFERENCES - APPENDIX

---

1. INTRODUCTION

This study is based on the work of researchers at the Ludwig Institute. These researchers have been studying the immunological mecanisms of cancer rejection for a few years. Recently, they have identified a gene that directs the expression of antigen MZ2.E on a human melanoma cell line (MZ2.MEL). This gene called MAGE 1 is expressed by other melanoma cell lines but no expression is observed in normal tissues. Antigen MZ2-E producted by MAGE 1 is recognized by C.T. lymphocytes only if it's presented by HLA. A1. (26% of total in Caucasian population). This human rejection antigen is shared by a significant proportion of other human tumours like breast cancer, small cells lung cancer, soft tissue tumours, respectively in 20%, 40% and 25%. The ability to identify these tumours readily on the basis of their expression of the relevant gene opens new possibilities for specific immunotherapy. Small tumours samples of HLA A1 patients can be frozen rapidly so as to ensure conservation of the RNA. This RNA can be tested by reverse transcription and PCR amplification to identify the tumors that express (gene) MAGE-1. Those tumors expressing antigen MAGE-1 may be sensitive to an CTL response. A study on the melanoma is now in progress. Here we propose a study for breast tumours in order to evaluate the CTL response.

2. OBJECTIVES OF THE STUDY

a) To assess the tolerance of a vaccine made of lethally irradiated allogeneic tumour cells on HLA-A1 patients whose tumour has been found to express MAGE-1.

b) To evaluate wether vaccination with allogeneic cells expressing MAGE-1 and HLA A1 increases the frequency of CTL directed against antigen MZ2-E

3. SELECTION OF PATIENTS

3.1. STAGE OF THE DISEASE

Because the most important aim of this study is to evaluate the CTL response after immunization, we suggest introducing :

a) Node negative premenopausal patients :

. Stage I T1 a ou b N0 M0

b) Node negative post-menopausal patients who are not eligible for protocol Tamoxifène/hautes doses MPA

. Stage I T1a-b N0 M0

. Stage IIA T2 N0 M0, if aged of > 70 ans at the time of diagnosis, not eligible for TMX-HDMPA

3.2. INCLUSION CRITERIA

To be elected, patients must fullfill all the following criteria :

1. HLA typing HLA.A1 : it is advisable to perform a complete HLA typing
2. Histologically confirmed diagnoses primary breast cancer and belonging to the categories described above
3. Patients should be in a stable general medical condition with performance status according to Karnofsky's scale 100-60, and a life expectancy of 3 months.
4. Age of 80 years or less
5. Patients must be able to comply with scheduled follow-up visit

3.3. EXCLUSION CRITERIA

1. Concomittant chemotherapy
2. Concomittant hormonotherapy
3. Psychosis or neurological alterations
4. History or evidence of allergic or autoimmune disease.

4. THERAPEUTIC SEQUENCE :

- Complete work-up
- Surgery : modified radical mastectomy or lumpectomy and axillary dissection
- Radiotherapy if indicated in all cases of conservative treatment
- Immunotherapy : the first immunization will not be sooner than 30 days after surgery, but may be concommitant of radiotherapy.
- Chemotherapy takes place after irradiation and immunotherapy.

5. IMMUNIZATION REGIMEN

Cells : Breast tumour cell lines will be used. These cell lines will be HLA-A1 and will have high expression of MAGE-1. The patients will therefore be immunized with cells that are allogeneic except for HLA-A1

Time course : Immunization will be carried out on days 0,30,60,120,180,300,420. For those patients who receive surgery, day 0 will not be sooner than 30 days after these treatment. Immunizations may be interrupted at the discretion of the investigator if major recurrence would occur. Most : each immunization will consist injections of 10^7 irradiated cells (one of each above mentioned cell lines). The cells will be irradiated at 10000 rads and will injected intradermally in the inguinal area where lymph drainage is not involved with the tumor site.

6. STUDY PARAMETERS

. Before the start of therapy : a complete medical history must be taken and a complete physical examination performed. Appropriate work-up must be performed including chest X ray or CT scan, mammography, liver ultrasonography or CT scan, bone scintigraphy, bone X ray .... The extent of the disease will be classified in accordance with the clinical staging system for breast cancer.

. Each patient undergoes a complete biological evaluation

- Haematological tests :
  - WBC
  - platelets
  - Hb
  - Haematocrit
- Special BC. tests :
  - CEA
  - CA 15.3
- Blood chemistry and liver function : Gamma GT, GOT, GPT, alkaline phosphatase, bilirubine
- Follow-up :
  - blood tests = every 6 months
  - extent Rx work-up :
    - every 6 months (the first year)
    - then every year